A. The present inventors conceived of this invention by considering how RecA and MutS work together "physiologically" in nature, when doing their "jobs" in safeguarding the fidelity of DNA replication and recombination *in vivo*. As stated in the specification.

> RecA molecules coat a stretch of ssDNA to form what is known as a RecA "filament." This filament, in the presence of ATP, searches for homologous sequences in dsDNA. When homology is located, a three stranded (D-loop) structure is formed wherein the RecA filament DNA is paired with the complementary strand of the duplex. If pairing is not perfect, *i.e.*, if there are mismatches or unpaired bases in the newly created duplex, MutS can bind to these structures and mobilize the other proteins of the mismatch repair system which act to abort the recombination event by removing the filament DNA and restoring the original duplex.... RecA binding to DNA may facilitate MutS mismatch recognition, perhaps by improving the presentation of mismatches to MutS.

The fundamental action of RecA recited in the above quote is exploited in various ingenious analytic methods in the prior art[2] (including that in the cited primary reference, Kigawa *et al., see below*). These are methods for identifying "genes" or specific sequences. Such methods are highly distinct from methods for detecting SNPs/mutations - to which the present claims are directed. **The fact that a method can be used to detect genes or specific sequences does not suggest the applicability of that method to SNP detection.** The differences in approaching these two different goals are significant. Hence, as is discussed below, the specific homology searching activity of RecA does not remotely suggest the present invention (namely, the use of MutS, *etc.*, in combination with RecA) nor does it suggest that the DNA structures formed by RecA *in vitro* might be substrates for MutS binding *in vitro*.

B. The present invention exploits a combination of RecA binding, to generate D-loops, and MutS binding to find mismatches or mispairings within the D loops, permitting the detection of SNP's and certain mutations. This method has many advantages over the inventors' own earlier invention of the use of immobilized MutS alone (as noted in the specification).

C. Because the present invention relies on MutS in the detection phase (a limitation in all the claims), it is important that the Office appreciate precisely what mutations (in addition to SNPs or point mutations) the claimed methods are capable of detecting. In fact, the only mutations

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[2] In fact, the use of RecA to identify or isolate specific sequences from complex DNA mixtures was proposed nearly 20 years ago by Charles Radding and colleagues (Honigberg *et al.*, *PNAS 83*:9586-9590, 1986)

that can be detected by the present methods are those that are detectable by MutS as a result of its inherent recognition capabilities. That was not stated literally in the specification (or claims) because it is obviously a "given" that this must be so. MutS, and its homologues in other bacterial genera and in eukaryotic cells, bind to nucleic acid duplexes having single base mismatches or from one to four unpaired bases. Thus, MutS is ideal for detecting SNPs and point mutations and also can detect mutations that involve an addition or deletion of about 1-4 nucleotides. See, for example, U.S. Pat. 6,027,877 (by Robert E. Wagner, Jr., one of the present inventors), as well as other of his cited patents and publications in the specification (See also Modrich, P. *J. Biol.Chem.264* 6597-6600, 1989 and Wu, F and Su, M., *J Biol Chem 272*:22714–22720, 1997, quotations from which are included in Appendix I. (Applicants will be happy to furnish these references if the Examiner wishes; the undersigned can e-mail pdf versions to the Examiner on very short notice if requested).

To reiterate this point: the present invention is geared specifically to identifying SNPs and a limited range of mutations that are recognizable by MutS when an appropriate ssDNA probe is provided that, by design, **differs**, *e.g.*, by one nucleotide, from the test DNA.

D. That being said, the present invention would not be of any value in a setting where (a) the test DNA and probe are perfectly matched, or (b) the two strands of ssDNA being allowed to anneal have homology of ***only*** between 90 and 95%. In the case of (a), MutS binding cannot occur. In the case of (b) either no binding will occur, or, if the "nonhomology" is distributed in a random manner over the length of the strands so that there are multiple, separate single base pair mismatches (or up to 4 unpaired bases), too much binding ("noise" or "false positives") will result, making the data uninterpretable.

E. The points in Paragraph D above are raised because the primary reference in the § 103 rejection, Kigawa *et al.*, describes a method that optimally seeks **perfect** homology (resulting in case 4a) or has as its lower limit of tolerance, 90-95% homology (resulting in case 4b). This is discussed further in the remarks directed to the specific rejection.

II. REJECTIONS UNDER 35 U.S.C. § 103

The Office Action sets forth grounds for rejecting the claims as obvious. For the reasons detailed below, Applicants respectfully submit that in light of the foregoing and following remarks, it would be appropriate to withdraw these grounds of rejection.

A. Legal Test for Nonobviousness

The burden of establishing a case of *prima facie* obviousness rests with the Patent and Trademark Office. *In re Fine*, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988). Moreover, an obviousness rejection "must be based on *evidence* (statutory prior art, admissions against interest)... ." *In re McKellin*, 188 USPQ 428, 432 (CCPA 1976), emphasis in original. The Federal Circuit Court of Appeals has repeatedly articulated the requirements of a proper analysis:

> [W]here claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under § 103 requires, inter alia, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. *See In re Dow Chemical Co.*, ... 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure.

In re Vaeck, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). It respectfully is submitted that a legally sufficient *prima facie* case of obviousness has not been adduced because the cited art does not suggest that the methods claimed be carried out with a reasonable expectation of success.

As respectfully noted below, the Action appears to focus on the obviousness of differences instead of on the claimed invention taken as a whole. This "is a legally improper way to simplify the often difficult determination of obviousness." *The Gillette Co. v. S.C. Johnson & Sons, Inc.*, 16 USPQ2d 1923, 1927 (Fed Cir. 1990), citing with approval, *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). In other words,

> It is wrong to use the patent in suit as a guide through the maze of prior art references, combining the right references in the right way so as to achieve the result of the claims in suit.

Orthopedic Equipment Company v. U.S., 217 USPQ 193, 199 (Fed. Cir. 1983). The nonobviousness of the claimed invention is discussed both above and below.

The hindsight assembling of the references cited against an applicant's claims, as the Office Action has done here, is legally improper according to the case law, such as *Orthopedic Equipment Co.* cited above. Moreover, once references have been chosen by an examiner:

> It is impermissible within the framework of § 103 to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art.

In re Wesslau, 147 USPQ 391, 393 (CCPA 1965). Thus, "prior art references ... must be read as a whole and consideration must be given where the references diverge and teach away from the claimed invention." See, for example, *Akzo NV v. International Trade Commission*, 1 USPQ2d 1241, 1246 (Fed. Cir. 1986).

B.. Specific Rejections and Discussion:

1. First Obviousness Rejection:

Claims 29-44 and 46-51 {*i.e.*, all claims but 45} are rejected under 35 U.S.C. 103(a) as being unpatentable over Kigawa *et al.*(U.S. Pat. 5,965,361, Oct 1999), hereinafter "Kigawa," in view of Nolan *et al.* (WO 99/22029, May 1999), hereinafter, "Nolan."

As stated in the Office Action, the primary reference, Kigawa, discloses the following:

(Note that several comments by Applicants are interspersed in a different font.)

(a) A method for detecting the presence of a double stranded (ds) target nucleic acid sequence using a probe/RecA complex (Abstract)

> ***[although the Office Action omitted the fact that detecting is in fixed cells or cell structures, not in solution].***

(b) use of a nucleic acid probe, typically single stranded nucleic acid, prepared by a virus, plasmid, or a cosmid, a probe DNA moiety excised from a vector, or probe from an oligonucleotide synthesizing method (which the Office relates to *instant claim 32*) (columns 5, lines 64-67 and column 6, lines 1-10).

> ***[A more precise characterization of col. 6, lines 1-10, which describes two alternative methods for making the probe, would be as follows:***

(1) a dsDNA restriction fragment is excised from DNA, run on a gel (electrophoresed), to isolate the dsDNA fragment, and then is subcloned into a ss vector (e.g., M13 phage), to get a ssDNA probe; or

(2) synthetically by synthesizing an oligonucleotide, and if necessary, ligating such oligos to one another to obtain a longer probe.]

(c) probes with **90-95% homology** to the target nucleic acid sequence and a length of 100 to 1500 bases (but may be <100 or >1500 nt's) (*related to instant claim 33*) (column 6, lines 12-18).

[Kigawa states that "90-95%" "definitely cause[s] a sequence-specific hybridization reaction" between probe and target. This is, in fact, the lower level of "tolerance" of the Kigawa approach.]

(d) The probe optionally may include a label, such as a fluorescent indicator, a radioactive label or a ligand that can be bound to a specific reporter molecule such as biotin and digoxigenin (*related to instant claim 34*) (column 6, lines 23-28).

(e) The RecA protein can include a detectable label or ligand, such as a fluorescent indicator, a chemiluminescent agent, an enzymatic label, a radioactive label, biotin or digoxigenin (*relates to instant claim 35-36, 39 and 41*) (column 6, lines 61-67).

(f) alternatively detecting the double-stranded target nucleic acid by allowing the probe/RecA complex to react with an anti-RecA antibody with or without a label or ligand (*relates to instant claim 40*) (column 10, lines 50-58).

(g) The hybridization reaction can be performed in the presence of another protein, such as SSB, if necessary to accelerate the reaction (*relates to instant claim 44*) (column 9, lines 18-22).

(h) The presence of the ds target sequence may be detected by fluorescence detection of fluorescently labeled RecA included in the probe/RecA complex bound to the target ds target sequence using a fluorescent microscope or flow cytometer *(relates to instant claim 42,43 and 46)* (column 10, lines 24-32).

(h) The probe/RecA hybridization method detects chromosomal aberrations such as deletions and insertions (column 13, lines 18-21).

[Note, however, that the types of deletions and insertions detectable by the Kigawa method are not the MutS-recognizable insertions or deletions of the present claims. It is highly improbable that the Kigawa method would permit

one to distinguish such small deletions/insertions (1-4 bases) from the wild type sequence.]

According to the Action, the only deficit in Kigawa as a reference in the absence of any teaching of the use of MutS protein with RecA for the detection of chromosomal aberrations.

[Kigawa also says nothing about using RecA for distinguishing SNP-containing sequences from "wild type" sequences. There are good reasons for such a "gap" in the Kigawa disclosure. Applicants want to be sure that the Examiner appreciates (as explained in more detail below) why there would be no motivation for a person of ordinary skill in the art who reads Kigawa and implements its methods for the purposes described in the reference, to consider the use of MutS. On the contrary, Kigawa "teaches away" from the use of MutS to "complement" or "supplement" or "improve" the Kigawa method, for a number of reasons set forth below.]

The **secondary reference**, Nolan, is cited for the following teachings:

(1) A method of detection of DNA polymorphisms including nucleotide polymorphisms, insertions, and deletions (page 1, line 6-7) that includes using microspheres to which a mismatch-binding protein (MBP) is immobilized, to bind to fluorescently labeled, mismatch-containing DNA by flow cytometry *(relating to instant claims 42-43, 46 and 48)* (page 4, lines 24-26) .

(2) In the above method, genomic DNA is amplified by PCR using fluorescently labeled nucleotide triphosphates *(relates to instant claims 31, 32 and 47)* (page 4, lines 26-28).

(3) MBP's include **bacterial MutS**, or any other protein that recognizes DNA base pair mismatches (and which can be immobilized on microspheres by physical absorption or by the use of an affinity tag which binds to an affinity partner immobilized on microspheres, such as biotin affinity tag and avidin/streptavidin binding partner) *(related to instant claim 34, 36-38, and 49-51)* (page 5, lines 23-29 and page 6 Table).

The Office concluded that it would have been *prima facie* obvious to **improve** the Kigawa method of detecting the ds target nucleic acid using a probe/RecA complex, **by including the MutS protein detection system** as taught by Nolan. According to the Office requisite **motivation** to improve the Kigawa method with immobilized MutS (Nolan) is found in the teaching by Nolan that the immobilized MutS immobilized system permits a *high throughput, small volume, and washless*

method for detecting SNPs in DNA (page 4, lines 5-6). The Office Action alleges that because of the rapid scanning of mismatched DNA per Nolan, the detection of RecA/probe complex formation as taught by Kigawa would be improved. As for the requisite "expectation of success," the Office alleges that there was a reasonable expectation of success that MutS could be used with the Kigawa method because of Nolan's teaching that use of MutS immobilized MutS for the detection of SNPs with flow cytometry provides multiparameter detection with excellent sensitivity in a homogenous assay format and multicolor fluorescent detection, and that this can be exploited for the simultaneous detection of dozens, or potentially hundreds, of analytes in a single sample (page 3, lines 9-14).

Applicants' Response

1. Although Kigawa mentions **detecting specific genes** (col. 13 para 2), determining chromosomal ploidy and even detecting chromosomal aberrations ("deletion, insertion, translocation, reversion, duplication and amplification"), it nowhere suggests that the method could ever be used to detect mutations as small as SNPs (or the slightly larger mispairings that MutS also recognizes). It is noteworthy that Kigawa prefers probes with sequence identity and will accept probes "with homology of at least 90% to 95% with the target nucleic acid sequence." **This teaches away from the present invention:** a person of ordinary skill in the art would appreciate that such homology levels would make impossible SNP/mutation detection using MutS (as claimed) or, in the best case, would generate false positives. In the present claims, the probe is designed to ***avoid*** successful homology searching (D-loop formation) unless there is nearly perfect homology (because this probe will generally form only a single nucleotide mismatch or unpaired base in the D-loop).

2. Kigawa fails the "suggesting" test in that it never suggests using oligonucleotides of different sequence than the target DNA to create an intentional mismatch. A mismatch would be an "anathema" to the Kigawa method. Rather, for obvious reasons, the Kigawa inventors strive to use probes that are identical to the target sequence they are attempting to detect. It is only logical that this reference does not suggest or motivate one to look to the MutS literature for any reason. On the contrary, this reference teaches away from that strategy. Kigawa never proposed use of any probe that would create a "substrate" for MutS because, again, the Kigawa method seeks to avoid mismatches. The goal is perfect pairing, which would render SNP or mutation detection

impossible. And the lower tolerance level called for in Kigawa ("at least 90-95% homology") would also render impossible SNP detection for reasons discussed in Sec. I.D, above.

3. The Kigawa method requires removal of the unreacted probes because they are labeled with the detectable label, and it is only that single label which is detected. The present method generates positive signal only when two (or more) components of the reaction are co-localized (*i.e.*, the oligonucleotide probe, MutS, and target DNA). In other words, in the claimed method, positive signal depends upon target DNA, MutS and RecA and/or oligonucleotide (as the carrier of the label). Unreacted components in the reaction mix are not scored.

4. The secondary reference (Nolan) does not fill any gaps left by Kigawa. There is no suggestion in Nolan (a) that the three stranded structure (the D-loop) formed by RecA homology searching could be a substrate for MutS binding, or (b) to combine the MutS method with any RecA-based method. Nolan used immobilized MutS to detect mismatches in duplex DNA. Furthermore, Nolan focused on fluorescently labeled target DNA and never suggested detecting or labeling the MutS (alone or in any combination). Applicants believe that the Office is using hindsight reconstruction in crafting the rejection, as it relies on the present inventors' teachings to pick and choose unrelated pieces of prior art to reconstruct the unobvious invention that Applicants have made. Such hindsight reconstruction is impermissible (*Orthopedic Equipment Company, supra).*

2. The Second Obviousness Rejection

Claim 45 was rejected as being obvious over Kigawa in view of Nolan as applied to claims 29-44 and 46-51 above, and further in view of Olson *et al.* (US Patent 5888728 March 1999) ("Olson"). The combination of Kigawa and Nolan (which Applicants have addressed and rebutted above), does not teach the use of detectably-labeled SSB protein. According to the Office, Olson fills this gap in its disclosure of streptavidin bound to biotinylated SSB in order for a SSB-oligonucleotide complex to bind to a capture membrane (column 5, lines 39-50 and 55-60). The Office concluded that it would have been *prima facie* obvious to improve the Kigawa method for detecting a mutation in a ds test DNA molecule with a probe/RecA complex bound to the test DNA,

(a) first as modified by Nolan, by contacting the DNA with MutS to detect the presence of MutS bound to the DNA structure, and

(b) second, as modified by Olsen by using a labeled SSB protein to improve the detection of the mutation in the target DNA.

The Office asserts that the ordinary artisan would have been motivated to improve the method of Kigawa combined with Nolan to include a labeled SSB protein because Olson teaches that the assay can be performed with "many combinations of sequential assays" (see column 4, lines 36-39). The Office went on to state that Olson teaches that many complexes may be formed, that these complexes may have one or more components, and that the assay can be designed to avoid interference from specific substances (column 4, lines 59-65). That allegedly provides the reasonable expectation of success when including a labeled SSB protein, in the method that combines Kigawa and Nolan.

Applicants' Response

Because the combination of Kigawa with Nolan does not give rise to an legally sufficient basis for a *prima facie* obviousness rejection, for the reasons stated above, the addition of yet another disconnected reference to that mix (Olsen) and its application against claim 45 does not cure the inadequacy of the primary or secondary references or their combination with respect to claim 45. Again, neither Kigawa, Nolan nor their combination can be said to suggest the use of SSB, or a need for anything like SSB. Olson does not suggest using SSB with D loop structures formed by RecA, alone, or with D-loops and mismatches recognized by MutS, to help detect SNPs or mutations. Again, there is no suggestions in these references to look to the others.

Summary of Applicants' Position on the § 103 Rejection

Based on the foregoing, Applicants believe that the primary reference cannot be considered an adequate legal basis for a *prima facie* obviousness rejection for reasons discussed above. It teaches away from the present invention and does not suggest looking to the secondary reference. Nolan certainly does not fill the gap left by Kigawa with respect to the present claims. Here too, there is no suggestion in Nolan to combine an immobilized MutS binding method with use of RecA filaments (D loops). Nolan uses immobilized MutS to detect mismatches in duplex DNA, but nowhere suggests that the three-stranded structure formed by RecA and dsDNA could be a substrate for MutS binding. Moreover Nolan never suggests detecting MutS, alone or in any combination (as only the probes are labeled). Finally, there is no suggestion in either of these references to consider using the SSB protein (Olsen). The Office can only have reached its conclusions that the claims are

obvious by improperly relying on Applicants' patent application "as a guide through the maze of prior art references, combining the right references in the right way so as to achieve the result of the claims..." (*Orthopedic Equipment Company, supra).* Since the Office has not met its legal burden under § 103(a), it would be proper to remove the rejections and pass all the pending claims to allowance.

III. CONCLUSION

In conclusion, it is respectfully requested that the above remarks and requests be considered and entered. Applicant respectfully submits that all the present claims are free of the prior art of record, and are therefore in condition for allowance. Applicants respectfully request early notice of such favorable action.

If these papers are not considered timely filed by the Patent and Trademark Office, then a petition is hereby made under 37 C.F.R. § 1.136, and any additional fees required under 37 C.F.R. § 1.136 for any necessary extension of time, or any other fees required to complete the filing of this response, may be charged to Deposit Account No. 50-0911. Please credit any overpayment to deposit Account No. 50-0911.

Respectfully submitted,

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APPENDIX 1

Modrich, P. *J. Biol.Chem.264* 6597-6600 (1989)

...Transfection analysis has also shown that the efficiency of methyl-directed repair depends on the nature of the mismatch, thus proving that mismatch recognition occurs during the course of the reaction. Of the eight possible base-base mispairs, the G-T and A-C transition mismatches and the G-G and A-A transversion mispairs are typically well corrected, while the T-T, C-T, and G-A transversion pairing errors are weaker substrates (18, 21, 25). The C-C transversion mismatch appears to be subject to little if any methyl-directed repair. The specificity of the methyl-directed system is not restricted to base-base mismatches because transfection experiments have also demonstrated efficient correction of mismatches involving insertion/deletion of a few nucleotides (20, 26).

20. Dohet, C., Wagner, R,, and Radman, M. (1986) *Proc, Natl*, Acad Sci. *USA*. 83:3395-3397
26. Fishel, R. A., Siegel, E. c., and Kolodner, R, 1986) *J. Mol. Biol.* 188, 147-157

W Fang, J Wu, and M. Su, *J Biol Chem 272*:22714–22720, 1997

...The mutator effects observed in *E. coli* ... mismatch repair-deficient mutants, are primarily transition and frameshift mutations (12). The fact that mutants deficient in mismatch repair show increased frequencies of frameshift mutations suggests that the *E. coli* mismatch repair system can recognize and repair heteroduplexes with one or more unpaired bases. Transfections of *E. coli* with artificially constructed heteroduplexes and *in vitro* assays have demonstrated that the different heterologies are subject to correction with different efficiencies. The methyl-directed repair of heteroduplex with one-, two-, and three-base deletions is as efficient as the repair of G-T mismatches (13–15). Heteroduplexes with a four-base deletion are marginally repaired, and DNA with a five-base deletion is not detectably repaired by the MutHLS system (15, 16)...

13. Dohet, C., Wagner, R., and Radman, M. (1986) *Proc. Natl. Acad. Sci. USA* 83, 3395–3397
14. Learn, B. A. *et al.*,. (1989) *J. Bacteriol.* 171, 6473–6481
15. Parker, B. *et al.*, (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89:1730–1734
16. Carraway, M. *et al.*,. (1993) *J. Bacteriol.* 175, 3972–3980